transformation. Reducing NSDs activity through specific and selective lysine-HMTase inhibitors appears promising to help suppressing cancer growth. In absence of ligand, the histone-binding site of NSD1 is occluded preventing any access to the catalytic groove. Therefore, we hypothesized that the SET domain of NSD1 has specific mechanisms to recognize histone marks unlike other HMTase.
Methods: We used computational methods to investigate the structural mechanisms happening in the SET domain during the binding of the H4-histone tail.
Results: Our finding exposes a key regulatory and a recognition mechanism driven by the flexibility of a loop at the interface of the SET and postSET region who rotates ~45° and translated 7Å opening the SET domain for the binding of the peptide ligand. This regulatory loop acts as a seat belt for the ligand and contributes to the discrimination and the substrate specificity. HMTase inhibitors are scare but our data bring significant insight into the design of specific and selective NSD-HMTase inhibitors.

225-Pos Board B11
High Level Expression of Proopiomelanocortin in *E. coli* Cells using Optimized Codons
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Proopiomelanocortin (POMC), a precursor protein, serves as the source of numerous biologically active peptides, including MSHs, ACTH, CLIP, LPH, and β-endorphin. POMC is processed at pairs of basic amino acid residues in a tissue-specific manner by pro-hormone convertase 1 and 2 (PC1/2). However, little is known concerning its own role and the mechanism associated with its processing, based on its tertiary structure. In addition, little information is available concerning the relation between the tertiary structures of the mature peptides and POMC.
In order to obtain structural information related to the processing mechanism of POMC, recombinant POMC was previously expressed using a T_7-promoter system in *E. coli* cells and the purified POMC was crystallized. However, the contaminating proteins affected the formation of POMC crystals. The expression level of POMC in *E. coli* cells was quite low, which made it difficult to eliminate contaminating proteins. Therefore, a system that permits the expression of much higher levels of the protein is required for the structural analysis of POMC.
For this purpose, the chemically synthesized cDNA encoding POMC, optimized at the codons and GC contents, was used to overcome the codon bias of *E. coli*. The PCR-amplified cDNA of POMC was introduced into several different expression vectors, such as pET17b and pPal7, and expressed in several types of *E. coli* cells. The recombinant POMC was well over-expressed in SHuffle T7 *E. coli* cells and purified by a combination of Ni-affinity and hydroxyapatite chromatography. The results will be discussed in this paper.

226-Pos Board B12
Crystal Structure of *E. coli* Tryptophanase in "Semi-Holo" Form: An Insight into Allostery of the Enzyme
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A new crystal form of *E. coli* tryptophanase (tryptophan indole-lyase, Trpase) (space group $P4_32_12$, a=b=109.97 Å, c=238.40 Å) was obtained under the same conditions as the tetragonal crystals of holo *E. coli*. Trpase. The structure was solved by molecular replacement at 3.2 Å resolution using the coordinates of apo *E. coli* Trpase (PDB code 2OQX) as a search model and refined to R=21.3 %, R_{free}=28.9 %. Out of two polypeptide chains contained in the asymmetric unit, one was found in holo form with PLP covalently attached to Lys271, while the other appeared to be in the apo form. The overall conformation of the holo subunit is the same as in the holo form of tyrosine phenol-lyase. The apo subunit is found in a wide-open conformation very similar to the one observed in the crystal structure of apo Trpase. Taking into account the flexibility of apo Trpase as seen in the known structures and difference in the crystallization conditions (pH, precipitant) and crystal packing, this finding is quite unexpected. We suggest that apo Trpase is found in the solution predominantly in the wide-open conformation which partially closes upon binding of PLP. The closed conformation might correspond to the enzyme state with both cofactor and substrate bound, in a way similar to tyrosine phenol-lyase. In addition, the conformation of the loop 301-310 is different in apo and holo subunits of the new structure suggesting that this conformational change is not induced by the oxidation of Cys298.

227-Pos Board B13
Effect of Crystal Packing on Cro Dimer Conformation
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X-ray crystallography is the primary method in structural biology for providing information about protein conformation. However, artificial packing forces in the crystal lattice select just a snapshot of a protein's conformational ensemble, whereas proteins are flexible and may adopt different conformations in order to function. This raises the question, how accurately do X-ray structures describe the solution state of a protein? To address this critical issue, we have established protocols for performing Molecular Dynamics (MD) simulation in solution as well as the crystalline environment and for using network analysis to study the conformational ensembles[1-3].
As a model system, we consider the λ Cro repressor, whose solved X-ray structures range from a closed to an open global conformation. The fully open form is observed both bound and unbound to DNA. Network analysis and a free energy surface constructed from Replica Exchange MD reveal that closed and semi-open conformations are stable in solution, with a modest barrier separating these two states[3]. Yet the fully open conformation, while accessible, lies higher in free energy, indicating it requires stabilization by DNA or crystal contacts. Since a semi-open state is among the low energy conformations sampled in simulation, we propose that this form may initiate DNA recognition and only minor adjustments are needed to achieve the fully open conformation as observed in the functional complex. Subsequent crystal MD simulations estimated the strength of packing interfaces in the lattice, showing the influence of crystal form and mutation in stabilizing different dimer conformations. Our quantitative results will aid analysis of X-ray data in establishing protein structure-function relationships. [1] Vorontsov, I. I. and Miyashita, O. (2009) *Biophys. J.*, **97**, 2532-2540. [2] Campbell, Z. T., et al. (2010) *Biophys. J.* **99**, 4012-4019. [3] Ahlstrom, L. S. and Miyashita, O., *submitted*.

228-Pos Board B14
Integrating Genomic Information with Molecular Simulation to Understand Protein Complex- and Active Conformation Formation in Two-Component Signal Transduction
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Protein function often requires a protein to form a complex or adopt multiple conformations during its functional cycle. Many of these states are transient or unstable and their full structural characterization remains a daunting experimental task. Here, we demonstrate a multi-disciplinary approach that can predict such structures for the common prokaryotic protein class of two-component signal transduction systems (TCS). TCS enable cells to sense and react to external stimuli. A membrane bound sensor histidine kinase (SK) detects an environmental stimulus and forms a complex with a transcription factor/response regulator (RR) transferring a phosphoryl group to mediate a cellular response. The complex is ruled by transient interactions. Despite decades of experimental studies, only few experimental structures are available: none of them trapped during autophosphorylation and only recently was a complex structure of a SK/RR pair structurally resolved [1]. Concurrently, we predicted this complex structure in high agreement (3.5 RMSD) with the experimental work by combining molecular dynamics and statistical genomic analysis [2,3]. Based on this theoretical work, it is now possible to also predict the structural changes occurring during autophosphorylation. Direct coupling analysis [3] identifies innerprotein pairings formed between the HisKa and ATP-binding domains which are not realized in the (inactive) crystal structure. This information can be used in molecular dynamics simulations to identify an active conformation adopted during autophosphorylation in agreement with biochemical mutagenesis data [4].
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